FBS28- PCR Amplification Using the GlobalFiler™ Kit

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1. Scope

1.1. This procedure is used to amplify DNA using the GlobalFiler™ PCR Amplification Kit.

2. Background

2.1. In order to ascertain if a profile(s) is present in a sample's extract, the purified DNA must be replicated and labeled for detection. The GlobalFiler™ PCR Amplification Kit is a short tandem repeat (STR) multiplex kit which utilizes the enzymatic process of Polymerase Chain Reaction (PCR) to amplify 24 specific DNA locations (loci). These 24 loci include the 20 core CODIS loci. Each kit is comprised of a fluorescent dye-labeled locus-specific Primer Set, PCR Master Mix (which includes the enzyme DNA polymerase), DNA Control 007 and Allelic Ladder.

3. Safety

3.1. Wear personal protective equipment (e.g., lab coat, gloves, mask, eye protection), when carrying out standard operating procedures (SOPs).

3.2. Read Safety Data Sheets (SDSs) to determine the safety hazards for chemicals and reagents used in the SOPs.

4. Materials Required

4.1. GlobalFiler™ PCR Amplification Kit – Primer Set and Master Mix with DNA polymerase enzyme

4.2. DNA Control 007

> **NOTE:** Thawing is required only during first use of the kit. After first use, reagents are stored at 2°C to 8°C and do not require subsequent thawing. Do not refreeze the reagents. Keep the kits protected from direct exposure to light. Excessive exposure can affect fluorescent probes. Each lot of kits must be evaluated prior to use. See FBQ41 for information regarding the procedure for evaluation. Amplification reagents must be stored separately from the evidentiary and reference samples.

4.3. 0.5 ml thin-walled reaction tubes (individual or strips)/strip caps or 96-well plate/strip caps/adhesive seal

4.4. TE (Tris EDTA) Buffer

4.5. Thermal Cycler – Applied Biosystems Gene Amp PCR System 9700

5. Standards and Controls

5.1. The amplification Positive (007) and Negative Controls are incorporated into the sample set following all other samples. Test at least one set of controls with each sample set (i.e., questioned and knowns).

5.1.1. DNA Control 007 is amplified as a Positive Control. This control is used to evaluate the performance of the amplification and subsequent typing procedures. See section 5.1 of the GlobalFiler™ Data Analysis Using GeneMapper® ID-X (FBS30) for the known profile that is generated from 007.

5.1.2. TE Buffer is amplified as the Negative Control. This control contains all of the chemical components of the amplification reaction in addition to TE Buffer and should exhibit no profile.

5.2. Extraction reagent blanks must be amplified at a volume equal to or higher than the highest preparation volume of any sample in its associated batch. In other words, the reagent blank should be amplified using the same concentration conditions as the forensic samples containing the least amount of DNA. This control contains all of the chemical components of both the extraction and amplification reactions and should exhibit no profile. Furthermore, the reagent blank must be amplified using the same primers and instrument model as the forensic sample(s) it is associated with.

5.3. If questioned and known samples will be amplified in the same 96-well plate, they must be separated by space (e.g., empty column) and questioned samples must be processed on the plate before known samples.

6. Procedures

6.1. Sample Set-up Calculations:

6.1.1. Using the estimated quantities of total DNA obtained from the Plexor® HY Quantification Kit, calculate the number of microliters (and/or the necessary dilution) to be added to the amplification reaction in order to obtain a concentration of approximately 0.05-0.10 ng/µL. The combined volume of TE buffer and sample DNA will equal 15 µL. A typical target amount of total DNA comprised within the 15 µL per validation procedures is 0.5 – 1.0 ng.

NOTE: The typical target amount listed above is based on single source samples and may be varied at the analysts' discretion based on the possible number of contributors in a sample.

6.1.2. The following chart is an example which can be used to calculate a sample to a 0.75 ng total DNA target:

If you are preparing the…	*Then…*
Total DNA sample and the concentration is ≤0.05 ng/µL	*Add 15 µL of sample to the PCR tube/well*
Total DNA sample and the concentration is > 0.05 ng/µL	*Dilute a portion of the sample with TE buffer so that only 0.75 ng of total DNA is in a volume of 15 µL.*

6.1.3. Record this information in the applicable Sample Tracking and Control Solutions (STACS) documentation.

6.2. Master Mix Preparation:

6.2.1. The amount of each component necessary to prepare the Master Mix/Primer mixture will be calculated upon entry of the number of reactions into the STACS amplification set-up.

# of Samples x 7.5 µL Master Mix

# of Samples x 2.5 µL Primer Set

NOTE: Extra reactions can be added to each calculation in order to account for volume lost during pipetting.

6.2.2. Plate set-up should be completed in the hood. Obtain the following components from refrigerated storage: Master Mix, Primer Set, and DNA Control 007.

6.2.3. Vortex and pulse spin, or tap on benchtop, all reagents. Record the appropriate lot numbers and expiration dates on the applicable STACS documentation.

6.2.4. Obtain a 1.5 ml or 2.0 ml tube and label as master mix. (If amplifying a large quantity of tubes, the master mix may be prepared in a V-bottom basin.)

6.2.5. Add the pre-determined amount of Master Mix and Primer Mix to the labeled master mix tube (or V-bottom basin).

6.2.6. Vortex and pulse spin if prepared in a tube. If prepared in a V-bottom basin, mix thoroughly (i.e. tip basin from side to side); however do not vortex. Store at 4°C until ready to aliquot.

6.3. Sample Distribution:

6.3.1. Allow the sample extracts to equilibrate to room temperature. Vortex and pulse spin all tubes.

6.3.2. **The order and labeling of the sample extract tubes must be witnessed by a second trained individual**. The witness step will be captured in the Batch Comments of the applicable STACS documentation.

6.3.3. Obtain and label an appropriate quantity of 0.5 ml thin-walled amplification tubes or a 96-well plate.

6.3.4. Place the tubes/plate in an appropriate retainer (tubes) and/or base (tubes and plate) for stability.

NOTE: The retainers should be used for sample transport from the pre-amplification laboratory to the post-amplification laboratory **ONLY**. Prior to re-entry to the pre-amplification laboratory, all retainers must be soaked in 10% bleach, rinsed with Deionized water (diH_20) and thoroughly dried. Alternatively, the retainers can be irradiated with UV light in a hood for 2 hours.

6.3.5. Aliquot 10 µL of master mix into each sample's amplification tube/well.

6.3.6. Following the STACS amplification set-up documentation, aliquot the calculated volume of neat extract/diluted extract and/or TE Buffer to each sample's associated tube or well. The DNA Control 007 and the TE Buffer Negative Control, in that order, will be the last two samples to be added to the batch. Aliquot an appropriate amount of DNA Control 007 and TE buffer to the Positive Control PCR tube/well for a total of 15 µL. Aliquot 15 µL of TE buffer to the Negative Control PCR tube/well.

6.3.7. All tubes/wells will now contain a total volume of 25 µL. Visually inspect the wells to ensure they contain a consistent volume.

6.3.8. Cap the tubes or cap/seal the tray. Make sure that the caps/seal are secure.

6.3.9. Vortex the amplification tubes/plate while in the base. Remove from the base and place the tubes/plate inside the pass-through for transport into the post-amplification laboratory.

NOTE: Do not place the base used during amplification setup into the pass-through. Empty bases are housed in the pass-through for purposes of holding the tubes/plate.

6.3.10. Once inside the post-amplification laboratory, retrieve the amplification tubes/plate from the pass-through area and centrifuge briefly.

6.4. Thermal Cycler:

6.4.1. Transport the amplification tray containing the tubes or the 96-well plate to the thermal cycler.

6.4.2. Load the samples onto the thermal cycler. Gently push the tubes/plate completely down into the heat block. Pull the lid closed over the samples until it clamps.

6.4.3. Turn on the thermal cycler. Select and start the “global-1” thermal cycling program. The method on the screen should correspond to the following:

HOLD	95°C	1 minutes
CYCLE	94°C	10 seconds
	59°C	90 seconds
Repeat for 29 total cycles		
HOLD	60°C	10 minutes
HOLD	4°C	forever

OPTIONAL: Thermal cycler may be preheated by turning on the thermal cycler anytime prior to adding tubes/plate to the thermal cycler.

6.4.4. Press Start.

6.4.5. When amplification is complete, the samples can sit at 4°C (in the thermal cycler) for up to 24 hours. Pulse spin tubes/plate after removal and freeze at

-20°C or proceed to preparation for analysis using the Capillary Electrophoresis Using the AB 3500/3500xl Genetic Analyzer SOP (FBS29).

7. Sampling

7.1. Not applicable

8. Calculations

8.1. The following chart is an example which can be used to calculate a sample to a 0.75 ng total DNA target:

Quant Value	*Action (Calculation)*
Initial concentration < Total DNA target	*Total DNA target / Initial concentration = volume to add to the amp (no dilution)* *Ex.: [0.75 ng / 0.05 ng/µL = 15 µL sample]* *Add 15 µL of sample to the PCR tube/well*
Initial concentration > Total DNA target	*(Initial concentration / Total DNA target) x Final amp volume = Dilution volume* *Ex.: [(1.5 ng/µL / 0.75 ng) x 1 µL = 2 µL (total) Dilution volume]* *Add 1 µL of 1:1 dilution to the PCR tube/well*

9. Uncertainty of Measurement

9.1. Not applicable.

10. Limitations

10.1. Given that the quantity reported by Real-Time PCR is an estimate, the amount of DNA added to an amplification reaction may be adjusted according to analyst experience/discretion or after detection to improve the quality of the profile.

10.2. For amplification troubleshooting procedures, refer to AB GlobalFiler™ PCR Amplification Kit User Guide. The following strategies can also be used:

Dilute DNA extract

Concentrate DNA extract

10.3. The fluorescent dyes attached to the primers are light-sensitive. Store all the samples away from light and minimize the time in which these samples are exposed during analysis.

11. Documentation

11.1. Applicable STACS documentation

12. References

12.1. Applied Biosystems. GlobalFiler™ PCR Amplification Kit User Guide

12.2. Applied Biosystems. GeneAmp® PCR System 9700 User Manual Set

12.3. Quantitation by Real-Time PCR Using Plexor® HY (FBS24)

12.4. Quality Control of GlobalFiler™ PCR Amplification Kit (FBQ41)

12.5. Capillary Electrophoresis Using the AB 3500/3500xl Genetic Analyzer (FBS29)

12.6. GlobalFiler™ Data Analysis Using GeneMapper® ID-X (FBS30)

12.7. Forensic Biology Unit Quality Assurance Manual